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THE APPLICATION OF A POTENTIAL-SENSITIVE CYANINE DYE TO RAT SMALL INTESTINAL BRUSH BORDER MEMBRANE VESICLES

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The sensitivity of the fluorescent dye, 3,3'-diethylthiadicarbocyanine (DiS-C_2(5)), was too low for the detection of membrane potential changes in rat small intestinal membrane vesicles. Only after adding $LaCl_3$ or after fractionation of the intestinal membranes by free-flow electrophoresis could the dye be used to monitor electrogenic Na^+-dependent transport systems. It is concluded that the response of this potential-sensitive dye is influenced by the negative surface charge density of the vesicles.

Potential-sensitive fluorescent dyes have proved to be a useful tool in membrane research (for review see Refs. 1–3). With cyanine dyes, changes in membrane potential were monitored during sodium-dependent, electrogenic substrate transport in renal brush border membrane vesicles [4–6].

Brush border membrane vesicles from small intestine and renal proximal tubules of male Wistar rats were isolated as described by Biber et al. [7]. For the separation with free-flow electrophoresis, the vesicles were suspended in 100 mM mannitol/20 mM Hepes-Tris (pH 7.4). The buffer used in the separation chamber was: 100 mM mannitol/10 mM triethanolamine/10 mM acetic acid, pH 7.4 with NaOH. The buffer used for the electrodes contained 100 mM triethanolamine/100 mM acetic acid, pH 7.4 with NaOH. The separation was performed with the Desaga FF 48 free flow apparatus (Desaga, Heidelberg, F.R.G.). The voltage was set to about 1020 V (130 V/cm) and the flow rate of the buffer in the separation chamber to about 180 ml/h (setting 3.5). The fractions were pooled (see bars on Fig. 3a), and washed with transport buffer (74 mM Na_2SO_4/1 mM K_2SO_4/20 mM Hepes/Tris (pH 7.4). Fluorescence measurements were performed as described in previous publications [5,8]. For each determination, 20 μl of membrane suspension (250 μg protein) were added to the cuvette. The ionic strength was kept constant in all buffers during the fluorescence measurements. All chemicals used were of analytical grade. DiS-C_2(5) was purchased from Eastman Kodak (Rochester NY, U.S.A.).

The addition of small intestinal brush border membrane vesicles to buffers containing 3 μM DiS-C_2(5) led to a decrease of the initial dye fluorescence (= 100%) to 32.9 ± 11.4% (15 preparations). This decrease occurred in the absence of an electrical potential difference ('resting fluorescence'). The same amount of renal brush border membrane vesicles decreased the fluorescence to 52.5 ± 5.7% (11 preparations). This finding suggests that more dye is associated with intestinal than with renal vesicles. Intestinal and renal brush

Abbreviations Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Dis-C_2(5), 3,3'-diethylthiadicarbocyanine iodide.

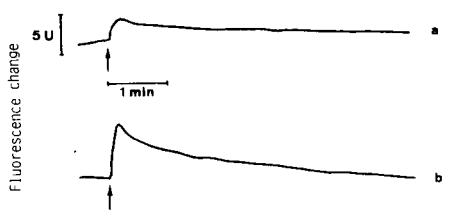

Fig. 1. Difference in the fluorescence signal after addition of D-glucose to intestinal and renal brush border membrane vesicles. Vesicles were loaded with 74 mM Na_2SO_4 1 mM K_2SO_4/20 mM Hepes-Tris (pH 7.4.). The buffer used for the incubation was the same. At the time marked by the arrow, D-glucose was injected into the cuvette with a syringe to a final concentration of 10.8 mM. (a) intestinal; (b) renal brush border membrane vesicles. After reaching the resting fluorescence level with a gain where fluorescence in the absence of vesicles was set to 100%, the gain of the apparatus was increased by a factor of 2.5.

border membrane vesicles differ with respect to the magnitude of D-glucose-dependent fluorescence signals (Fig. 1). A fluorescence increase after addition of D-glucose to vesicles indicates an inside-positive electrical potential difference due to electrogenic sodium-dependent uptake of D-glucose (Beck and Sacktor [4]). The corresponding D-glucose signal is considerably greater in renal vesicles (Fig. 1). When equal potassium diffusion potentials were imposed on renal and intestinal vesicles, again a greater fluorescence change was found with renal brush border membrane vesicles (not shown). This suggests different quantitative responses of the dye in measuring changes in membrane potential in rat intestinal and rat renal brush border membrane vesicles, respectively, rather than monitoring different transport rates. Fig. 2a shows a saturable increase in the resting fluorescence after addition of $LaCl_3$ to the buffer. This indicated that dye was released from intestinal vesicles into the buffer. In contrast to intestinal vesicles, addition of $LaCl_3$ to renal vesicles did not increase the resting fluorescence (data not shown). Lanthanum increased the fluorescence changes following the addition of D-glucose, D-galactose or L-alanine (Fig. 2b). In renal vesicles, lanthanum had no effect on D-glucose dependent fluorescence changes (data not shown). In order to exclude an effect of lanthanum on the transport rate, uptake experiments with radioactively labeled D-glucose in the presence and absence of $LaCl_3$

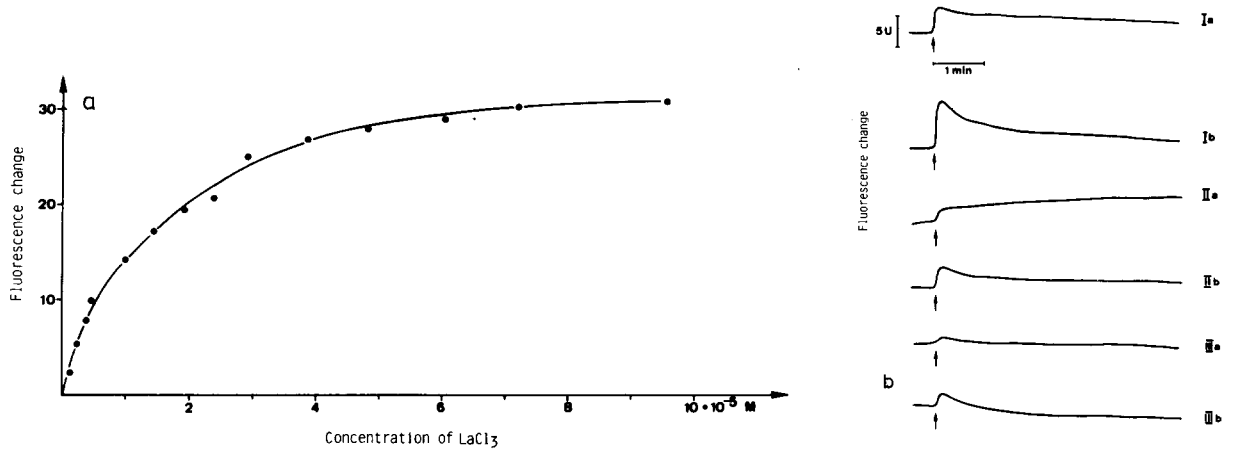

Fig. 2. a. Increase in resting fluorescence in dependence on $LaCl_3$ concentration. Intestinal vesicles loaded with the same buffer as used for the experiment in Fig.1 were added to an identical buffer containing the dye. 0.5 min later, $LaCl_3$ was added to the final concentrations indicated on the abscissa. After the fluorescence had reached a new steady state, the difference to the fluorescence in the absence of $LaCl_3$ was determined and depicted on the ordinate. Fluorescence in the absence of vesicles was set to 100%. The effect of lanthanum on the increase of 'resting' fluorescence was measured without changing gain. b. Influence of $LaCl_3$ on fluorescence signals of different transport substrates. Intestinal vesicles were loaded and suspended in the same buffer as used in the experiment for Fig. 1. At the time indicated by the arrow, the substrate was injected into the cuvette with a syringe to a final concentration of 10.8 mM. Substrates: I, D-glucose; II, D-galactose; III, L-alanine. (a) is a signal in the absence and (b) a signal in the presence of $9.71 \cdot 10^{-6}$ M $LaCl_3$ (resting fluorescence in the absence of $LaCl_3$: 58, in the presence of $LaCl_3$: 103). As in Fig. 1, the gain was increased by a factor of 2.5.

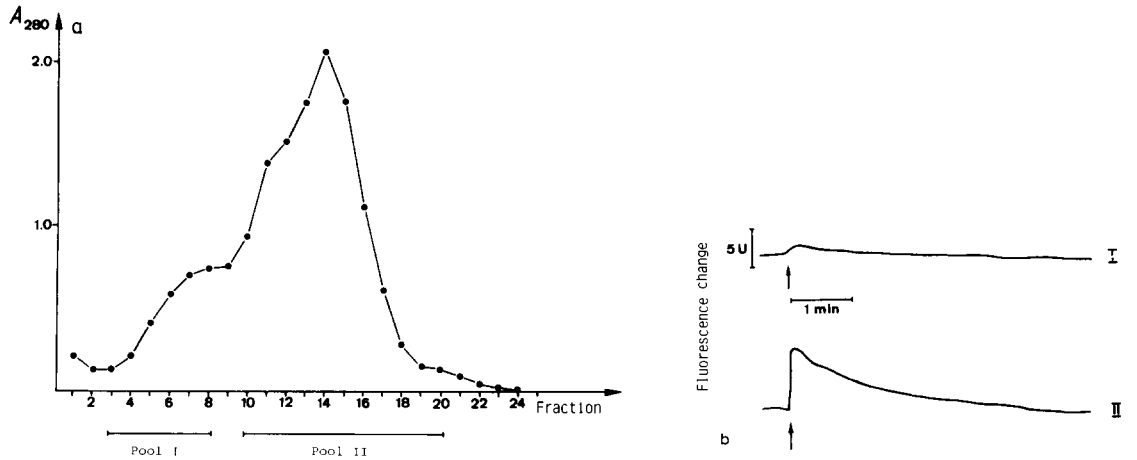

Fig. 3. a. Distribution pattern of jejunal brush border membrane vesicles after free flow electrophoresis. The buffer system used was the same as described in Fig. 1. The separation conditions were: 1030 V (147 V/cm), 115 mA buffer flow: 158 ml/h (setting 3); $t = +3.5$°C. b. Comparison of fluorescence signal of two vesicle pools I and II after free-flow electrophoresis. Intestinal vesicles were loaded and suspended in the same buffer as in the experiment for Fig. 1. At the time marked by the arrow, D-glucose was injected into the cuvette with a syringe to a final concentration of 10.8 mM. I, vesicles from the left pool (resting fluorescence: 81); II, vesicles from the right pool (resting fluorescence: 123). As in Fig. 1, the gain was increased by a factor of 2.5.

were performed (data not shown). As 10 μm $LaCl_3$ slightly inhibited sodium-dependent tracer-D-glucose uptake into renal and intestinal brush border membrane vesicles (data not shown), the increased fluorescence signals in the presence of $LaCl_3$ cannot be due to an increased transport rate.

In order to investigate the influence of the surface charges, we subjected rat jejunal vesicles to free flow electrophoresis. A distribution pattern of these vesicles after the fractionation is given in Fig. 3a. When vesicles (250 μg protein) from pool I were added to buffers containing 3 μM DiS-$C_2(5)$, the fluorescence dropped to a steady level of $34.8 \pm 6.5\%$ (ten preparations). Vesicles from pool II (250 μg protein) decreased the fluorescence to a steady level of $48.8 \pm 5.0\%$ (ten preparations), indicating less dye binding to membrane vesicles obtained from pool II (cathodic side of the electrophoresis). As seen in Fig. 3b, 3 μM DiS-$C_2(5)$ responded to the addition of 10.8 mM D-glucose much better with vesicles derived from pool II than with vesicles from pool I. With vesicles from pool II, the fluorescence changes of the dye reflected the properties of the D-glucose transport system with respect to sodium dependency, stereo- and substrate specificity and saturability (data not shown).

In conclusion, our findings indicate an influence of surface charges of membranes on the sensitivity of the dye to inside-positive membrane potentials. A high negative surface charge density is associated with a low resting fluorescence, and low sensitivity of the dye. By screening negative surface charges or by further separation of the vesicles by free-flow electrophoresis, we extended the application of the cyanine dye to rat small intestinal brush border membrane vesicles.

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